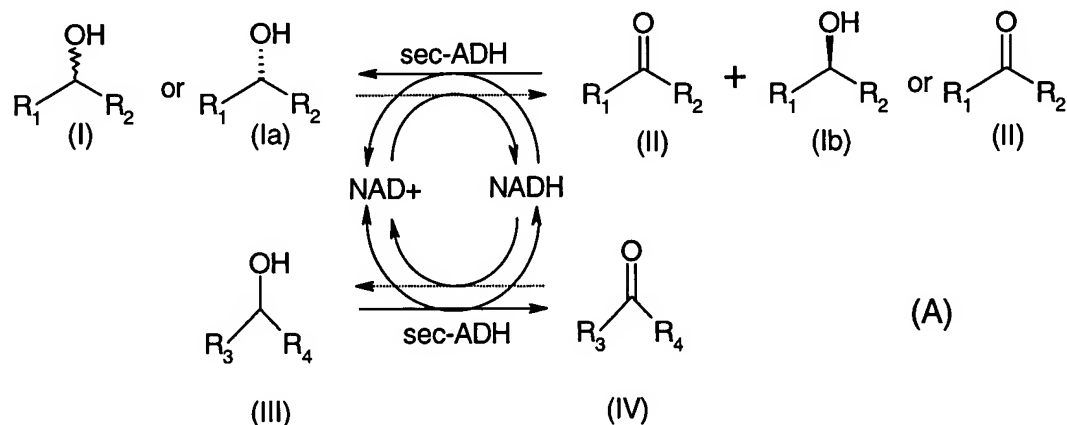


Claims:

1. A biocatalyst having alcohol dehydrogenase activity which can be obtained from *Rhodococcus*.
2. The biocatalyst according to claim 1, obtainable from *Rhodococcus ruber* DSM 14855
3. The biocatalyst according to claim 1 which shows stereospecific alcohol dehydrogenase activity in the oxidation of secondary alcohols or the reduction of ketones.
4. The biocatalyst according to claim 1 in purified form.
5. The biocatalyst according to any one of claim 1 having a molecular weight on denaturing SDS polyacrylamide electrophoresis from 32 to 44 kDa, especially of about 38 kDa.
6. The biocatalyst according to claim 1 having a molecular weight according to size exclusion chromatography of 55 to 69 kDa, especially of about 62 kDa.
7. The biocatalyst according to claim 1 comprising  $Zn^{2+}$  bound to the molecule.
8. The biocatalyst according to claims 1 including the partial sequence EVGADAAAR (SEQ ID No: 1) within the total sequence of at least one polypeptide forming the whole or part of the enzyme, or wherein one of the amino acids mentioned in the partial sequence is exchanged against a different amino acid.
9. The biocatalyst according to claim 1 including the partial sequence TD[L/I]FEVVA[L/I]AR (SEQ ID NO: 2) within the total sequence of at least one polypeptide forming the whole or part of the enzyme, where [L/I] is leucine or isoleucine, or wherein one of the amino acids mentioned in the partial sequence is exchanged against a different amino acid.
10. The biocatalyst according to claim 1 including the partial sequence SGAGAADA[L/I]R (SEQ ID NO: 3) within the total sequence of at least one polypeptide forming the whole or part of the enzyme, where [L/I] is leucine or isoleucine, or wherein one of the amino acids mentioned in the partial sequence is exchanged against a different amino acid.
11. The biocatalyst according to claim 1 including the partial sequence V[L/I]AVD[L/I]DDDE (SEQ ID NO: 4) within the total sequence of at least one polypeptide forming the whole

or part of the enzyme, where [L/I] is leucine or isoleucine, or wherein one of the amino acids mentioned in the partial sequence is exchanged against a different amino acid.

12. The biocatalyst according to claim 1 including the partial sequence V[L/I]AVD[L/I]DDDXRX? (SEQ ID NO: 5) within the total sequence of at least one polypeptide forming the whole or part of the enzyme, where [L/I] is leucine or isoleucine and X stands for an unidentified amino acid, or wherein one of the amino acids mentioned in the partial sequence is exchanged against a different amino acid..
13. The biocatalyst according to claim 1 including the partial sequence sequence [TD/DT] [L/I]MEVVA[L/I]AR (SEQ ID NO: 6, either with TD in the beginning or with DT in the beginning) within the total sequence of at least one polypeptide forming the whole or part of the enzyme where the sequence in brackets is selected from the two alternatives mentioned therein and where [L/I] is leucine or isoleucine, or wherein one of the amino acids mentioned in the partial sequence is exchanged against a different amino acid.
14. A recombinant biocatalyst according to claim 1.
15. A biocatalyst according to claim 1, which shows alcohol dehydrogenase activity in the presence of up to 50, preferably up to 80 percent by volume of isopropanol, or in the presence of up to 20, preferably up to 50 percent by volume of acetone.
16. The use of a biocatalyst with alcohol dehydrogenase activity according to claim 1 in the oxidation of secondary alcohols and/or the reduction of ketones.
17. The use according to claim 15, where the oxidation and/or reduction is one of the reactions in the following reaction scheme (A)



wherein, in formula I, Ia and Ib,  $R_1$  and  $R_2$  are two different moieties from the group consisting of unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkynyl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted aryl and unsubstituted or substituted heterocyclyl, or  $R_1$  and  $R_2$  together form an unsubstituted or substituted bridge; and in formula III and formula IV,  $R_3$  and  $R_4$  are two different or preferably two identical lower alkyl or aryl moieties, or together form a bridge.

18. The use according to claim 15 in the stereoselective production of chiral or diastereomeric alcohols from the corresponding ketones.
19. The use according to claim 16 in the stereoselective production of chiral or diastereomeric alcohols from the corresponding ketones.
20. The use according to claim 15 in the chemoselective, especially stereoselective, oxidation of secondary alcohols where only the hydroxy group with the appropriate steric form is oxidized to the corresponding oxo group
21. The use according to claim 18 where the stereoselective oxidation is used for the enantioselective oxidation of only one isomer of a mixture of enantiomers or diastereomers of secondary alcohols.
22. A nucleic acid coding for a biocatalyst with alcohol dehydrogenase activity according to claim 1.
23. A nucleic acid according to claim 22, especially a DNA or RNA, which is a recombinant nucleic acid.
24. A microorganism transformed with a nucleic acid coding for a biocatalyst according to claim 1.
25. The use of a microorganism according to claim 24 in the production of a biocatalyst mentioned in claim 24.

26. The use of a microorganism, especially a host cell expressing the biocatalyst, according to claim 24 in the catalysis of the oxidation of secondary alcohols or the reduction of ketones.

27. . A polynucleotide, namely a DNA, or a recombinant polynucleotide each comprising a DNA, each coding for an enzyme showing alcohol dehydrogenase activity, where the DNA is characterized by the following sequence (SEQ ID NO: 47)

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ATGAAAGCCG TCCAGTACAC CGAGATCGGC TCCGAGCCGG TCGTTGTCTGA CATCCCCACC 60
CCGACGCCCCG GGCCGGGTGA GATCCTGCTG AAGGTCACCG CGGCCGGGCT GTGCCACTCG 120
GACATCTTCG TGATGGACAT GCCGGCGGCG CAGTACGCCT ACGGCCTGCC GCTCACCTTC 180
GGCCACGAGG GTGTCGGCAC CGTCGCCGAA CTCGGCGAGG GCGTCACGGG ATTCGGGGTG 240
GGGGACGCCG TCGCCGTGTA CGGGCCGTGG GGCTGCGGTG CGTGCCACGC CTGCGCGCGC 300
GGCCGGGAGA ACTACTGCAC CCGCGCCGCC GACCTGGGCA TCACGCCACC CGGTCTCGGC 360
TCGCCCCGAT CGATGGCCGA GTACATGATC GTCGATTTCG CGCGCCACCT CGTCCCGATC 420
GGAGACCTCG ACCCGGTTCG CGCGGCGCCG CTCACCGACG CCGGTCTGAC GCCGTACCAC 480
GCGATCTCCC GGGTCCTGCC GCTGCTGGGG CCGGGCTCGA CGGCCGTCGT CATCGGTGTC 540
GGCGGGCTCG GCCACGTTCG CATCCAGATC CTGCGCGCCG TCAGCGCGGC CCGTGTGATC 600
GCCGTTCGACC TCGACGACGA CCGTCTCGCC CTCGCCCCCG AGGTCGGCGC CGACGCGGCG 660
GTGAAGTCGG GCGCCGGTGC GGCGGACGCG ATCCGGGAAC TGACCGGCGG CCAGGGCGCG 720
ACGGCGGTGT TCGACTTCGT CGGCGCCCAG TCGACGATCG ACACGGCGCA GCAGGTGGTC 780
GCGGTCGACG GGCACATCTC GGTCTGTTGG ATCCACGCCG GCGCACACGC CAAGGTCGGG 840
TTCTTCATGA TCCCGTTTCG CGCCTCCGTC GTGACCCCGT ACTGGGGCAC CCGGTCGGAA 900
CTGATGGAGG TCGTCGCGCT GGCCCGCGCC GGCCGGCTGG ACATCCACAC CGAGACGTTT 960
ACCCTCGACG AGGGGCCGGC GGCCTACCGG CGGCTGCGCG AGGGCAGCAT CCGCGGCCGC 1020
GGCGTGGTGG TTCCCTGA 1038
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or variants thereof with conservative nucleic acid replacements that lead to no change in the resulting amino acid sequence or such changes in the base sequence that lead to amino acid replacements in the amino acid sequence resulting from translation thereof for 5 % or less of the total number of amino acids coded by and resulting from translation of the sequence of SEQ ID NO: 47, provided that the resulting polypeptides still display an alcohol dehydrogenase activity, and/or with insertions and/or deletions.

28. . A polynucleotide or a recombinant polynucleotide according to claim 27 translation of which results in 3 % or less amino acid replacements compared with those resulting from the nucleotide sequence mentioned in claim 27 as SEQ ID NO: 47
29. .A polynucleotide or a recombinant polynucleotide according to claim 27 comprising a DNA of SEQ ID NO: 47.
30. A polynucleotide or a recombinant polynucleotide according to claim 27 that codes for a polypeptide with alcohol dehydrogenase activity that has at least one of the following properties:
- pH optimum in the reduction of ketones in the presence of NADH: pH 6 to pH 7;
  - pH optimum in the oxidation of alcohols, especially of 1-phenylethanol in the presence of NAD<sup>+</sup>: pH 8.5 to pH 9.5;
  - temperature optimum in the reduction of ketones in the presence of NADH: between 43 and 65 °C;
  - temperature optimum in the oxidation of secondary alcohols in the presence of NAD<sup>+</sup>: between 43 and 65 °C;
  - temperature stability under the conditions just given for the temperature optimum at 50 °C less than 35 % activity loss during 24 hours;
  - stability also in the presence of up to 50 percent by volume of isopropanol;
  - stability also in the presence of up to 20 percent by volume of acetone.
31. .A. polynucleotide or a recombinant polynucleotide according to claim 30 that codes for a polypeptide with alcohol dehydrogenase activity that has all of the properties given in claim 30.
32. .A DNA complementary to the polynucleotide or recombinant polynucleotide in claim 27.
33. A vector comprising a recombinant polynucleotide according to claim 27.
34. A vector according to claim 33 wherein the polynucleotide comprises a DNA with SEQ ID NO: 47.
35. A microorganism transformed with a polynucleotide or a recombinant polynucleotide according to claim 27.
36. A microorganism transformed with a vector according to claim 33.

37. A polypeptide showing alcohohol dehydrogenase activity of the sequence with SEQ ID NO: 48:

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MKAVQYTEIG SEPVVVDIPT PTPGPGEILL KVTAAGLCHS DIFVMDMPAA QYAYGLPLTL 60
GHEGVGTVAE LGEGVTGFGV GDAVAVYGPW GCGACHACAR GRENYCTRAA DLGITPPGLG 120
SPGSMAEYMI VDSARHLVPI GDLDPVAAAP LTDAGLTPYH AISRVLPLLG PGSTAVVIGV 180
GGLGHVGIQI LRAVSAARVI AVDLDDDDRLA LAREVGADAA VKSGAGAADA IRELTGGQGA 240
TAVFDFVGAQ STIDTAQQVV AVDGHISVVG IHAGAHAKVG FFMIPFGASV VTPYWGTRSE 300
LMEVVALARA GRLDIHTETF TLDEGPAAAYR RLREGSIRGR GVVVP 345
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as well as truncated or elongated versions thereof or those with amino acid replacements of up to 5 % of the number of amino acids forming part of the sequence given under SEQ ID NO: 48 and/or insertions, sequence extensions or deletions or combinations of two or more thereof, provided that the resulting polypeptide still displays an alcohol dehydrogenase activity.

38. A polypeptide according to claim 37 with the sequence given as SEQ ID NO: 48 or a version thereof wherein up to three percent of the amino acids are replaced by other amino acids..

39. A polypeptide according to claim 37 with the sequence given as SEQ ID NO: 48 or a version thereof wherein up to 3 amino acids are replaced by other amino acids.

40. A polypeptide according to claim 37 with the sequence of SEQ ID NO: 48

41. A polypeptide according to claim 37 that has at least one of the following properties:

- pH optimum in the reduction of ketones in the presence of NADH: pH 6 to pH 7;
- pH optimum in the oxidation of alcohols, especially of 1-phenylethanol in the presence of NAD<sup>+</sup>: pH 8.5 to pH 9.5;
- temperature optimum in the reduction of ketones in the presence of NADH: between 43 and 65 °C;
- temperature optimum in the oxidation of secondary alcohols in the presence of NAD<sup>+</sup>: between 43 and 65 °C;
- temperature stability under the conditions just given for the temperature optimum at 50 °C less than 35 % activity loss during 24 hours;
- stability also in the presence of up to 50 percent by volume of isopropanol;

- stability also in the presence of up to 20 percent by volume of acetone.

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42. A. polypeptide according to claim 41 with alcohol dehydrogenase activity that has all of the properties given in claim 41.
43. A method of using a polypeptide according to claim 37 for the oxidation of secondary alcohols and/or the reduction of ketones, comprising administering the reaction educts and substrates and the polypeptide in purified or partially purified form to a reaction medium and performing the oxidation and/or reduction.
44. A method of using a microorganism according to claim 35 for the oxidation of secondary alcohols and/or the reduction of ketones, comprising administering the reaction educts and substrates and the microorganism in complete or digested form to a reaction medium and performing the oxidation and/or reduction.